CHAPTER – 2

REVIEW OF LITERATURE
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Man depends on natural ecosystems to meet his basic physical needs for clean air and water, food, waste assimilation, medicinal compounds, and for many less tangible needs like outdoor recreation and aesthetic beauty to renew our spirits (Daily et al., 1997). Comprehensive studies have also shown that healthy environments generally support healthy economics (Hall, 1994; USEPA, 1996) and protection of all existing species at large (Meyer, 1998). Despite such significant human dependence and concerns for the natural environment, compelling scientific evidence indicates that current human use and allocation of natural resources are clearly threatening both the ecological and social sustainability of our planet (Karr and Chu, 1995; Karr and Chu, 1999; Brown et al., 1997).

More than one billion people today lack access to an adequate supply of safe water for household use. In 30 years, as many as 5.5 billion people may live in areas suffering from moderate to severe pressure on water resources, rendering the provision of safe water even more difficult. In light of these trends, new approaches are urgently needed to manage water resources rationally and equitably (Newson, 1994; Newman, 1995; Das, et al., 2001).

The explosion of human population and industrial activities, and the rate at which new chemicals and products are being developed and used pose a global environmental threat. The natural decay process in water bodies can no longer cope with these loads (Ananthi et al., 2004) as thousands of water based industries have come up during the last three-four decades (Tiwari, 1995; Petts and Amoros, 1996; Karl et al., 1997).

Water pollution is a serious problem for the entire world. It threatens the health and well being of humans, plants and animals. Since water plays such a vital role in the life on earth, good quality of water is a precious resource. Often, water quality is more important than water quantity (Pimentel et al., 1997; Howsen, 1997; Karr, 1999). Implementation of
water pollution prevention strategies and restoration of ecological systems are integral components of all development plans. To preserve our water and environment, we need systematic changes in the way we grow our food, manufacture the goods, and dispose off the waste (Kelly and Harwell, 1990; Harris, 1995; Lazaroff, 2000).

Environmental improvement and restoration should be planned and implemented such that the freshwater resources are protected and their quality is maintained or enhanced. Management of river water quality has become increasingly important due to decline in water quality caused by human activities (Jolma, 1997; Karr and Chu, 1999; Rakesh et al., 2005).

2.1 Water quality status

Of all the renewable resources, water has a unique place. It is essential for sustaining all forms of life, food production, economic development, and for general well being. It is impossible to substitute for most of its uses, difficult to depollute, expensive to transport, and it is truly a unique gift to mankind from nature.

Water quality study is an integral part of evaluation of health of any wetland ecosystem. In general, the riverine ecosystem, while traversing through hills, before reaching plains, maintain unpolluted water quality due to less human activities and can be regarded as good and potable water and suitable for sustenance of aquatic life (Das and Sinha, 1993; Sahu et al., 1994; Stormer et al., 1996; Evans et al., 1999; Leonard, 2002; Huertas et al., 2002; Jensen, 2003).

Poor water quality caused by pollution and lack of habitat diversity are often the limiting factors in developing successful fisheries management programmes in rivers. The effects of pollution may be very subtle and indirect (Hoffmann and Parsons, 1991; Tully et al., 1993; Cognetti, 1994; Birkeland, 1996; Nolan et al., 1999; Arrignon, 1999; Dufresne et al., 2002).
With the rapid industrialization and urbanization during the past 50 years, most of our rivers have been subjected to indiscriminate discharge of effluents affecting water quality and aquatic life (Verma and Shukla, 1969; Ambasht, 1990; Binkley and Brown, 1993; Nevo et al., 2001). Rivers are used in various ways, such as for fish capture, irrigation, to procure fish seeds and also as beds for the disposal of the industrial wastes of different factories and domestic wastes of cities (Verma et al., 1978, 1980; Ashutosh et al., 1993; Hossetti et al., 1994; Karl et al., 1997). The indiscriminate disposal of industrial waste into natural water system has created a number of hazards to the aquatic life (Mathur, 1965; Rajaguru and Subburam, 2000; Agarwal, 2002; Rakesh et al., 2005).

Sahu (1991) and Allan (1995) have revealed the various factors that changed the physico-chemical characteristics of river water. The aquatic pollution by the addition of many of the toxic and deleterious materials to the water bodies causes adverse effects in water quality. The industries are mostly situated on riverbanks or other water bodies, as they require plenty of water for their production purposes. In industrial activities, innumerable new chemicals are being introduced, the by-products of which are discharged into the aquatic environment as wastes (Cunningham et al., 2005). The indiscriminate use of pesticides has also severely affected the water quality (Narayanasamy, 1999).

Singh (1995) has linked the fish mortality to the depletion of oxygen and high content of total ammonia with alkaline pH in the river water due to influx of sugar factory effluent and subsequent high values of BOD, COD, total ammonia and pH. Dutta et al. (1997) have indicated that fish mortality occurred due to sudden discharge of deoxygenated industrial effluents containing H2S and very high free CO2 and BOD.

Konar (1981) and Pandey et al. (2000 a, b) have excellently reviewed the literature describing the effects of pollutants and fish-kill in polluted waters. Rounsfall and Everhart (1950) have pointed out that even when the pollutants do not kill or seriously harm the fish, the changes produced in their biological environment such as destruction of their food and habitat indirectly affect the fish population.
The physico-chemical environment of water functions in one or more ways and influences the biotic components. Thus it gives a picture of the environmental suitability of water for maintaining normal life (Kumar, 1997). Pollution of river associated with industrial discharge and refuse from human settlement is a global problem. In India it has been reported that about 70 percent of the available water is polluted (Citizen's Report, 1982). Urbanization and industrialization lead to the generation of large volumes of wastewater from domestic, commercial, industrial and other sources (Bremmer and Keeney 1966; Vimal and Talashilkar, 1985). Ajmal and Khan (1985), Higgins and Desher (1986), Jones and Case (1990) and Peng and Cassman (1994) have reported that large amounts of heavy metals along with other toxic chemicals are discharged as industrial wastewater.

Fish is widely recognized as an excellent source of protein of very high quality. The rapidly increasing use of pesticides, chemicals and fertilizers in agriculture pose a serious threat to fin fishes. Increasing pollution of rivers and other water bodies have become a matter of great concern in recent past. Further, the accumulated toxicants in fishes may reach human being as they form a staple food (Barton and Iwama, 1991; Schreck, 2000; Rotllant et al., 1997).

### 2.2 Water Quality of river Periyar

All over the world, the rivers in high altitude are subjected to exploitation to generate hydel energy resulting disturbance in the riverine ecosystem. The study of water quality of rivers in India are well documented by Ghatak and Kumar, 1992; Jameson and Rana, 1996; Abbasi et al., 1996; Gyanath, 2000; Baruah et al., 2003.

Water quality of river Periyar has been investigated particularly in its lower reaches by Jayapalan et al. (1976), Paul and Pillai (1978, 1986), Devi et al. (1979) and Joseph et al. (1984). Sankaranarayanan and Quasim (1969) investigated the nutrient status of Cochin backwaters at the region of river discharge and reported that during monsoon the concentration of nutrients is quite high in the estuary especially in the bottom, which they
reasoned, is due to river discharge and decomposition of organic matter in the bottom sediments. Nutrient enrichment is the main reason for eutrophication, which leads to algal bloom and consequent BOD increase in water bodies.

The studies conducted by several investigators revealed that Periyar water is polluted due to effluent discharge from various industrial installations situated on the banks of the river (Joseph et al., 1984; Sankaranarayanan et al., 1989; Joy, 1989). Ramani et al. (1980) observed fluctuations in the composition and nature of sediments caused by industrial effluents discharged into the river.

Devi et al. (1979) also have reported that the industrial effluents released into Periyar at the Eloor industrial zone affects the hydrographical features during the pre-monsoon and post-monsoon months. Jayapalan et al. (1976) and Joy (1989) observed that during summer the river water is characterized by low dissolved oxygen, high temperature, low pH, high ammoniacal nitrogen, phosphate, less phytoplankton diversity and high chloride content, while during monsoon it possesses high dissolved oxygen, low temperature, high carbon dioxide content, normal pH and low chloride.

Silas and Pillai (1976) and Shynamma et al. (1981) have reported fish mortality in the river. The pollution profile of the river Periyar, as represented by Paul and Pillai (1978, 1986) revealed high concentrations of pollutants such as Ra$^{228}$, PO$_4$, Zn and Mn in the water sediment even at location 2 km down stream of the industrial outfalls. Balakrishnan and Devi (1983) highlighted the increasing environmental problems in the river Periyar and adjoining Cochin backwater system due to industrial effluents. The dams across the river, often several of them, along the course have affected the flow pattern, the quality of water, riverine biodiversity, the extent and the nature of sediment formation and deposition (Lakshmanan et al., 1987).
2.3 Physico - chemical Stress in river Periyar

It has been reported that the water quality of the river is considerably altered during pre-monsoon so that there is occasionally increase in temperature, lowering of pH, dissolved oxygen and high core of nutrients such as nitrite, ammonia and phosphate (Nair et al., 1976). Joy et al. (1990a, b) have reported that the water flow in Periyar during summer months is insufficient to effect dilution of waste water received in the industrial zone. Devi et al. (1979) has also reported elevated temperature at the industrial discharge area of river Periyar in the non-monsoon months. Unnithan (1979) found erratic fluctuations of pH during non-monsoon months in the industrial zone. The lowering of pH could be due to acidic effluents discharged from the industries at this location. Silas and Pillai (1976) have reported large-scale mortality of fishes in the industrial belt of river Periyar, which was attributed to highly acidic water.

Turbidity affects aquatic forms, as the bottom conditions such as light penetration, bacterial concentration and several other factors are usually modified by the amount of suspended particles in the water. The coarse suspended particles settle down very quickly and original clear water condition is restored to some extent after a distance of several kilometers from the discharge place of the effluent (David, 1956; Sreenivasan and Sundraraj, 1967; Ghosh and Basu, 1968; Arora et al., 1973). David and Ray (1966) have noted that excess of dissolved solids may create an imbalance and sudden change in the osmoregulation or cause suffocation of fish.

The river is reduced to a narrow stream of clear shallow water during the summer months of April and May. Increased turbidity and saline water incursion form Cochin backwaters affect the water quality. Clay bed scouring in the river sides affects the water quality which renders the water unfit for industrial, agricultural and drinking purposes and leads to casualties due to the creation of ditches in the river (Gaur and Kumar, 1981). During summer (April, May), the riverbed is exposed at many locations. In June, with the onset of southwest monsoon, the water level rises and the water turns muddy and reddish.
brown in color due to land run off (Joy, 1997). The turbidity at the point of sewage openings is, however, considerably higher on accounts of sewage discharge and the high turbidity checks the phytoplankton growth in the drain (Chandra, 1980).

The salinity incursion reaches about 15 km upstream during pre-monsoon and post-monsoon period. The influx of salinity occurred upto Edayar during pre-monsoon where the salinity ranged between 0.44 and 9.78 ppm (Joy, 1989). However, Sankaranarayanan et al. (1986) have reported salinity upto a distance of 25 km from Cochin harbour mouth.

In the Eloor – Kalamassery industrial belt, the effluent directly discharged into the Periyar river from where it enters the backwaters. Major chemical pollutants present in these waters are acids, alkalies, suspended solids, fluorides, free ammonia, ammoniacal nitrogen, insecticides, dyes, chromium, Hg, Zn, other metals and an unknown quantity of radioactive nuclides, Thorium and Uranium (Stephan, 1985). Taking advantage of the topography and the tidal influence of the waters, the effluents in these factories are dumped into the Periyar at certain locations. Owing to almost regular discharge of a variety of wastes, the Periyar river bottom in the vicinity of the industrial belt is completely covered with decaying organic matter (Azis and Nair, 1981). Devi et al. (1979) studied the water quality of the industrial region. They also assessed some of the characteristics of the effluents discharged into the river and found that the level of ammonia, a potent toxin, in the effluents and water is high. Temperature and variable pH along with occasional high chloride and COD levels especially in the dry months seem to make this environment hazardous. The monsoonal floods provide adequate dilution and mask the effect.

Many hazardous substances including heavy metals, discharged into the aquatic environment are known to accumulate in the estuarine sediments. Ramani et al. (1980) studied the levels of Cu, Mn, Co, Ni and Zn in the sediments of the northern limb of Cochin backwaters, which runs through industrial belt. All metals showed some degree of variations over the area studied. Co showed the lowest variation, Cu and Zn values vary
with stations and seasons. The main effluent discharge site showed significant enrichment in Cu during monsoon and in Zn during pre-monsoon. Bar mouth sediments showed an increase in metal content in post-monsoon.

Chemical speciation of a metal, together with reactions involved in its transformation, is often the factor determining diversity of the pollutant in aquatic environment. Shibu et al. (1990) revealed that speciation of metals in the Cochin estuary is influenced by environmental factors such as (a) influx of riverine input of metals regulated by dams /reservoirs, (b) introduction of industrial effluents and sewage, (c) modifications arising from anthropogenic activities and (d) hydrographic changes related to complexity of water use. The metals Cu, Co, Ni, Zn and Cd show high affinity to Fe and Mn, which establishes the importance of Fe and Mn hydroxides /oxides in the distribution of trace metals (Jayasree and Nair, 1995). The impact of these industrial effluents is often experienced in the estuary as mass mortality of the fish, *Ambassis ephalus* as reported by Unnithan (1977).

The heavy metal estimation revealed the localized concentration of certain heavy metals especially Cd, Co, Zn and Cr in the vicinity of Eloor industrial belt as well as adjacent regions of north Vembanad estuary (Jenne, 1968). The major sources of iron are effluents of industries connected with iron or steel and units in which iron is one of the raw materials (Gopinathan et al., 1974).

**2.4 Stress on Fishes**

The factors that can cause stress are called stressors. In aquatic environment the physical stressors are temperature, light, suspended and dissolved materials. Chemical stressors leading to poor water quality includes low dissolved oxygen, improper pH, accumulation of various pollutants etc. Biological stressors constitute crowding, aggression, territoriality, pathogenic and non-pathogenic organisms etc.
When a fish (or any other animal) is under environmental stress, it will experience internal changes that are either detrimental or adaptive. It cause changes in cellular function, which then alter the physiology of organ systems. Such alterations in organ functions may, if not corrected or compensated for, weaken the fish so that it is less able to cope with other stressors. Reduced reproductive potential may result, thereby affecting the structure and function of entire fish communities or ecosystems (Alan, 1990). Adams (1990) has listed the details under which a fish or a population of fish is either under stress or not under stress.

An organism is continuously subjected to environmental stresses of different kinds due to changes in environmental factors, either diurnally or seasonally. The stress can induce damage in a living system and if allowed to accumulate with time, ultimately leads to death. Besides, a living system is distinguished from a non-living organization by an innate characteristic feature of ‘stress resistance’ or ‘homeostasis’. This is manifested either as ‘stress avoidance’ or ‘stress tolerance’ (Prosser, 1958). While the former is the capacity to exclude the stress partially or completely (by Claude Bernard’s constancy of the ‘milieu interior’), the latter is the capacity of survival with a thermodynamic equilibrium between the internal and the external environment of an organism (Mallat, 1985; Andersan, 1990; Thomas, 1990).

In all cases the level of stress induced by specific factor is highly species-dependent. Changed growth can be the result of physiological stress caused by the physico-chemical environment. Physiological stress could alter enzyme and hormonal levels in fish, which could cause cell or tissue damage, which further could affect the ability to produce vital offspring (Lehitnen, 1990).

Chronic stress, whether from pollutants or other factors, generally reduces the ability of fish to resist pathogenic organisms, to adjust physiologically or behaviourally to other physical stressors such as water currents or predators, and to reproduce. The
immune system that functions in the defense against pathogen is generally affected by stress (Jiminiz and Stegeman, 1990).

Chronic stresses associated with intensive fish farming include such phenomena as deterioration of water quality (Smart, 1981) and the less obvious but equally important stresses of social interaction. The very act of rearing fish at unnaturally high stoking densities may increase the problem of social dominance, with submissive fish showing enhanced internal activity, reduced growth rate and increased susceptibility to infections (Schreck, 1981). The pressures of industrial requirements and the recreational demands upon aquatic ecosystems have resulted in biological stress at all levels of organization, not least with respect to fish populations. Important researches have been undertaken to determine lethal limits when fishes are subjected to deleterious changes in the environment as a direct result of man’s activities (Elliott, 1981).

2.5 Physico-chemical Stressors

2.5.1 Temperature

Temperature is a prominent physical stressor. It exerts important influences on the immune system of fish. Temperature stress, particularly cold temperatures, can completely halt the activity of “killer cells” of the immune system, thus, eliminating an important first defense against invading organisms; excessively hot temperatures are also very detrimental to fish, although the precise impact of sudden increases in temperature on the immune system is not known (Wanger et al., 1997).

Ambient temperature is a critical factor in the development of both specific and non-specific host immunity (Watts and Burke, 2001) and any change in temperature can affect the immune response (Carlson et al., 2002). In general, regardless of the fish species examined, elevated water temperature that still remains within the physiological range of the species has been shown to alter immune function (Bly et al., 1997). For example, exposure of catfish (Heteropneustes fossilis) to elevated temperature increases
mitochondrial superoxide production in the gills and enhances antibody activity in Atlantic cod. Esteban et al. (2001) demonstrated that exposure of Atlantic halibut to increased water temperature had no effect on phagocytosis, but reduced host resistance against bacterial challenge. In *Cyprinus carpio* the haematocrit value and haemoglobin level grew with increasing water temperature; marked changes were recorded when water temperature increased to $35^\circ$C. The mean corpuscular haemoglobin concentration remained almost unchanged at temperatures of $20^\circ$C and $25^\circ$C. At $35^\circ$C it increased by 7% (Turosik, 1986).

Seasonal changes in haemoglobin concentration and erythrocyte dimensions of *Lepomis microlophus* were consistent with a pattern of increasing values with increasing water temperature. Erythrocyte size in *Cichlasoma cyanoguttatum* decreased with increasing water temperature. Hemoglobin concentration of the two species was similar, but *L. microlophus* had a large number of erythrocytes and less hemoglobin per erythrocyte than *C. cyanoguttatum* (Atkinson and Judd, 1978).

The biochemical and molecular strategies of adaptation are best known with respect to the thermal stress. This is because temperature is by far the most pervasive of all environmental factors, which influence the biological activities of organisms in our planet. A slight increase in temperature can produce a large degree of augmentation in the rate of most of the functions in an organism. The majority of organisms being thermo conformers or poikilothermic, cannot demonstrate ‘stress avoidance’. Rapid temperature change is a major cause of physiological stress in fish (Zelikoff et al., 1995).

### 2.5.2 pH

Acidification of freshwater bodies has been widely observed in the recent years in the vicinity of industrial belts of our country (and abroad) due to the discharge of various effluents from the factories that affects aquatic biota adversely (Beaman et al., 1999).
2.5.3 Dissolved Oxygen

Oxygen consumption plays a major role in the physiology of fish. It is also an index of its metabolic rate. Various factors like size, temperature (Savitz, 1968; Vivekanandan and Pandian, 1977; Jobling, 1980), stocking density (Kjartarson et al., 1988), seasonal variation (Chanchal and Prasad, 1992), physiological factors (Patel and Patnaik, 1990) and starvation influence oxygen consumption in fish. Besides this, various constituents of medium also affect oxygen consumption in fish. Chemicals like urea, ammonia (Sarkar, 1991; Amutha and Mahalingam, 1999), Copper (Satyanarayana and Balaji, 1991) and Cadmium (Fromm and Gillete, 1968; Reddy et al., 1993; Davidraj and Jesily, 1996) are also known to influence oxygen consumption of fish.

Fish absorb oxygen directly from the water into their blood stream using their gills. Oxygen depletion is the cause of many fish kills. Oxygen stress is a common cause of disease outbreaks. The amount of oxygen in water decreases as temperature and altitude increases. As temperatures increases, fish metabolism will increase and hence they consume more oxygen. So both the increase in temperature and fish metabolism may cause oxygen depletion in the summer (Moly, 2000).

2.5.4 Free Carbon dioxide

The harmful effects of CO2 on fish have usually been associated with reduction in oxygen affinity and oxygen capacity of the blood (Alabaster et al., 1957; Basu, 1956; Saunders, 1962). The physiological responses to exposure have also been extensively studied and give some indication of how fish are able to acclimate to elevated levels of CO2. The examination of fish from a number of fish farms with high CO2 concentrations in the water supply has revealed the presence of nephrocalcinosis. The severity of the condition at a particular CO2 concentration does appear to vary greatly according to diet and environmental factors such as ionic content (Landolt, 1975).
2.5.5 Nitrate

It is an important toxicant to freshwater fish even when it is present in the environment in a relatively low concentration. This anion is an intermediate product in bacterial nitrification and denitrification processes in ecosystems. Under normal conditions, free ammonia is transformed by bacterial oxidation (Nitrosomonas sp.) to nitrite and then to nitrate (Nitrobacter sp.). However, an imbalance of this oxidation system, or a high rate of ammonia production can result into the disruption of the nitrification process and increment of environmental nitrite, thus resulting in a toxicity episode caused by this anion, which can be of special importance in habitats receiving nitrogenous effluents, in hypoxic environments, and in intensive aquaculture systems with unbalanced water filtering (Eddy and Williams, 1987; Tomasso, 1994; Jensen, 2003).

Nitrate is considered as a disrupter of multiple physiological functions, including ion regulatory, respiratory, cardiovascular, and excretory processes. The first consequence of nitrate entrance is the formation of methaemoglobin (met Hb), thus causing functional anaemia (Eddy, 1983; Tomasso, 1994; Aggergaard and Jensen, 2001; Huertas et al., 2002). However, different studies have reported that the primary cause of nitrite toxicity can be different for each fish species (Doblander and Lackner, 1997; Huertas et al., 2002; Jensen, 2003), suggesting that the toxicity of nitrite results from a combination of effects rather than from any single effect in particular (Jensen, 2003).

2.5.6 Salinity

Siberian sturgeon is hyperosmotic to its environment and requires an active uptake of ions across the gills in order to osmoregulate and compensate their passive loss across
the gill epithelia and urine (Rodryquez et al., 2002). Since nitrate is actively taken up across the gills in competition with chloride (Evans et al., 1999; Fontentot et al., 1999), when this toxicant is present in the environment, an ionic imbalance resulting from a significant increase in plasma chloride and potassium levels is observed in Siberian sturgeon. This increase in plasma Cl (hyperchloremia) has been reported by Huertas et al. (2002) as a stress factor in fishes.

The assessment of fish ionic regulation status may be done via the analysis of plasmatic parameters which offers the potential for several parameters to be sensitive to toxicant stress: cortisol, ion concentration, glycemia, hormones, haemoglobin, and haematocrit (Hlavova, 1993; Perry et al., 1996; Luskova, 1998). Among them, Cl content has been shown to be particularly useful for studying the physiological disturbances in fish caused by naturally or accidentally modified environmental factors (Musselman et al., 1995; Madsen et al., 1996; Zydlewski and McCormick, 1997). Some blood parameters, such as Cl content have also proved to be quiet sensitive toward many toxicants in laboratory experiments (Lauren and McDonald, 1985; Poleo et al., 1995; Patrick and Wood, 1999). However, little information is available about their potential application under field conditions, particularly in free-living fish populations.

A wide range of environmental stresses can be the reason of physiological failures (Goss et al., 1992; Wendelaar and Lock, 1992; Rosseland and Staurnes, 1994; Truchot, 1994; Roche and Boge, 1996). Most studies dealing with fish regulation in hypersaline waters have focused on adaptation of fish to estuarine or sea conditions, especially during migrations (Ouseph et al., 1994; Zydlewski and McCormick, 1997; Handeland et al., 1998).

2.5.7 Heavy metals

Metals are present in very low concentrations in natural aquatic ecosystems (Morehead et al., 2000), usually at the nanogram to microgram per litre level, but recently the occurrence of especially heavy metals in excess of natural loads, has become an
increasing concern (Patino and Thomas, 1990; Monder and White, 1993; Pankhurst and Dedual, 1994; Pankhurst, 1995; Van Der Kraak, 1997; Pankhurst and Van Der Kraak, 1997; Mommsen, 1999; Schreck, 2000) for aquatic ecosystem.

Heavy metals have been recognized as strong biological poisons because of their persistent nature, toxicity, tendency to accumulate in organisms and undergo food chain amplification (Monder and White, 1993; McMaster, 1994; McCormick et al., 1998; McEwen, 1998; Pankhurst, 1998; Morehead et al., 2000). They also damage the aquatic fauna including fish. The fishes were affected, either directly through uptake from the water, or indirectly through their diet, vegetation, invertebrates or smaller fish (Slater and Fitzpatrick, 1995; Sumpter, 1997; Schulz and Goose, 1999; Schreck et al., 2000).

Metals released into aquatic ecosystems, are responsible for the physiological irregularities of fishes (Khan et al., 1991; Zhao and Chou, 1993; Katakura and Sugawara 1995; Lauwerys et al., 1995). All of these effects of heavy metals usually affect fishes negatively leading to stress and eventually, in most cases, death (Marks et al., 1996; Nishiyama et al., 1998; Novelli et al., 1998; Nriagu, 1998; McFarland et al., 1999; Barbosa and Ribas, 2000).

Heavy metals are one class of pollutants, which have a disruptive influence on the structural organization of the gill tissue. Because the gills are intimately associated with ionic regulation it is predictable that heavy metals will influence aspects of osmotic and ionic regulation in fish. With relatively high levels of Zn^{2+} (40ppm.) rainbow trout die mainly through tissue hypoxia (Skidmore, 1970; Burton, et al., 1972), a major factor being disruption of the brachial respiratory epithelium (Skidmore and Tovell, 1972). Skidmore (1970) found little change in the arterial blood plasma osmotic pressure or ionic content of rainbow trout after Zn^{2+} treatment whereas Lewis and Lewis (1971), using rather lower Zn^{2+} concentrations over a longer period of time, found significant decreases in the plasma osmotic pressure of channel catfish, *Ictalurus punctatus*, and this was considered a major factor leading to death (Stagg and Shuttleworth, 1981).
The anaemia induced may be due to the suppression of haemopoietic tissues by the accumulation of heavy metal (Kumada et al., 1980) and the defect in iron metabolism caused by deficiency in intestinal absorption (Richardon et al., 1974). It has been widely reported in fishes that many pollutants especially metals enter the RBC and either oxidise or denature Hb by inhibiting the glycolysis or metabolism of the hexose monophosphate shunt (HMPS) (Kumada et al., 1980; Mattsson et al., 2001; Nunes et al., 2001).

Larger but sub-lethal levels of Zn\(^{2+}\) can induce pathological and morphological abnormalities in adult fish and acute exposure to zinc can also cause an impaired branchial calcium influx leading to hypercalcemia in fishes (Dunson, 1970, 1972; Camerone, 1976; Swift and Lloyd, 1974; Proenca and Bittencourt, 1994; Peters and Livingstone, 1996; Papas, 1996; Oteiza et al., 1997; Papas et al., 1998).

2.6 Haematology and Stress studies

Haematological features are widely used as effective tools to assess the stress responses in fishes. The most common haematological variables measured during stress include, red blood cell count, white blood cell count, haemoglobin, hematocrit, mean corpuscular haemoglobin etc. Water quality should be considered as one of the major factors responsible for individual variations in haematology, because fish live in close association with their external environment and are sensitive to any change that may occur within their environment (Casillas and Smith, 1977). Changes in osmoregulation in fish exposed to some stressors are generally elucidated by measuring the blood plasma sodium, potassium, calcium, chloride and the total osmolarity (Heath, 1977). The sodium, potassium and chloride ions are responsible for the preservation of the osmolarity and crystalloid osmotic pressure of the plasma (Meyer, 1988). Regulation of the plasma ion concentrations is accomplished by the gills, kidneys and some special organs and to some extent, by the integument in its role as a barrier (Bond, 1979). After short term exposures, at both temperatures, there were no significant changes in the plasma sodium.
concentration. However, in contrast to this, there were significant decreases in the plasma sodium concentration at both temperatures, following long-term exposures.

McCarthy et al. (1973) and Hughes (1989) have elaborated the importance of haematology in the diagnosis of diseases in fishes with respect to pollution. Chemical analysis of blood could also reveal various aspects in relation to different diet and diseases. Studies on the impact of physico-chemical factors on the haematology of lower vertebrates showed that in all the reported studies there is a close correlation between the haematological values and eco-physiological conditions (Srivastava and Pandey, 1986).

Variation in haemoglobin can be considered as a response of fishes to changing environmental condition and therefore, it may be used to assess the health of fishes exposed to toxicant (Gill and Pant, 1981). The adaptability of the organism, to frequent changes in environmental conditions is studied from the release of RBCs into the blood from those stored in spleen (Toft, 1955).

According to Mattson et al. (2001) higher numbers of WBC and thrombocytes were present in fish exposed to untreated effluent. In fish exposed to treated effluent, lower values of MCH and MCV were also observed by Murty and Hanke (1985). The effect of sublethal concentration of urea on the haematological parameters such as RBC, WBC, Hb, PCV, MCH, MCV and MCHC of the air breathing fish Anabas testudineus (Bloch) has been investigated (Vindimian et al., 1991). Conspicuous fall of haematological parameters were observed in the fertilizer treated fish even though an increase in the total erythrocyte count was recorded (Bhatt and Singh, 1986; Phillip et al., 1994; Dhanapakiam and Ramasamy, 2001).

2.6.1 pH

Variations in pH is found to cause osmotic disturbances causing change in the shape of blood cells, and other haematological aspects (Hughes and Umezawa, 1962; Thomas and Hughes, 1981; Leifestad, 1984; Wood, 1988; Witters et al., 1990; Sayer,
1991; Witters, 1991; McCormick and Jensen, 2003; Collins and Brown, 1999; Laitinen and Valtonen, 1995; Nair and Suryanarayanan, 2000; Dussault, 2001).

2.6.2 Oxygen

The physiological and behavioural alterations that occur in fishes in the absence of sufficient oxygen have been investigated. In all cases, the erythrocytes showed karyorrhexis and a unique form of cell division (Chavin, 1973; Schreck et al., 1976; Wedemeyer et al., 1976; George, 1977; Mazeaud et al., 1977). Hypoxia caused significant but transient increase in hemoglobin, haematocrit and mean corpuscular haemoglobin concentration (Malte, 1986; Qureshi et al., 1995; Lim et al., 2000; Cole et al., 2001; Lohner et al., 2001; Mattsson et al., 2001; Affonso et al., 2002; Carlson and Parsons, 2003).

2.6.3 Heavy metals

Aluminium

The impact of exposure to increased concentrations of Aluminium on fish has been studied. Aluminium toxicity is found to cause ion regulatory imbalance, increased blood viscosity, decrease in erythrocytes, lower haematocrit, swelling of erythrocytes and irregular blood cell count (Witters, 1986; Sayer, 1991; Witters, 1991; Poleo and Muniz, 1993; Laitinen and Valtonen, 1995; Collins and Brown, 1999; Allin and Wilson, 1999; Dussault, 2001).

Iron

Deficiency in iron leads to anaemic conditions in fishes, but high amount of iron has been found undesirable and subsequent changes in haematological parameters were found to be statistically significant (Firdaus et al., 1994; Naser et al., 1980).

Cadmium

Koyama and Ozaki (1984) observed an initial decrease in haemoglobin concentration and an increase in the WBC count in Cyprinus carpio exposed to cadmium, while Houstan and Keen (1984) observed a fall in the RBC count and hemoglobin
concentration in the goldfish, *Carassius anratus* when exposed to sub lethal concentration of cadmium. Other workers (Gupta, 1979; Panigrahi and Misra, 1980; Agarwal *et al.*, 1982; Gill and Pande, 1983; Aruna and Gopal, 1995; Goel *et al.*, 1985 and Goel and Sharma, 1987) have obtained similar results in other fishes by exposing these to different heavy metals.

Chronically sub lethal concentrations of cadmium caused conspicuous hematological anomalies in the cyprinid fish, *P. conchonius*. Exposure to 0.63 and 0.84 mg/l cadmium chloride induced morphological aberrations in mature erythrocytes including cytoplasmic vacuolation, hypochromia, deterioration of cellular membrane, basophilic stippling of cytoplasm, clumping of chromatin material, extrusion of nuclei and schistocytosis. The mean corpuscular haemoglobin and mean corpuscular volume increased but mean corpuscular haemoglobin concentration showed no obvious change (Lauwerys *et al.*, 1995). A significant thrombocytopenia, elevated small lymphocyte and basophil populations, and a mild neutropenia were manifested in the cadmium - exposed fish (Zhao and Chou, 1993). Large lymphocytes were not significantly affected (Marks *et al.*, 1996). Blood parameters of *Cyprinus carpio* exposed to acute sub lethal concentrations of cadmium nitrate (24 ppm) and mercuric chloride (0.30 ppm) for 90 hours resulted in significant decrease in erythrocyte count, hematocrit and hemoglobin content. Hepatosomatic index of the fish exposed to these metallic pollutants increased (Gill and Pant, 1985; Beena and Viswaranjan, 1987; Hymavathi and Rao, 1999).

It has been reported that the cadmium - loaded fish suffered from a slight anemia and a disturbed carbohydrate metabolism, blood plasma ion composition and higher lymphocyte count (Sjoebeck, 1984; Gill and Epple 1993). Studies of Witeska and Baka (2002) also exposed the deleterious haematological changes when common carp were treated with cadmium. In fish, cadmium has adverse effects on growth and reproduction and causes osmoregulatory stress, and it was shown to alter the structure and function of various organs, including liver. Studies on the impact of cadmium on metabolism are
scarce. Particularly, the specific effects of cadmium on energy-producing metabolic pathways in fishes have received little attention (Koyama and Ozaki, 1984; Yamawaki et al., 1986; Sastry and Shukla, 1994; Soengas et al., 1996; Witeska and Baka, 2002).

**Zinc**

Bioaccumulation of zinc was estimated in freshwater fishes viz. *Labeo rohita, Catla catla, Channa punctatus* and *Poecilia reticulata* after acute and sublethal exposure to different concentrations of this metal and zinc smelter effluent (Madhusudan et al., 2003). Exposure to sub lethal Zinc concentrations has found to decrease the red blood cell counts, haemoglobin, haematocrit and mean corpuscular volume, which can be ascribed to anaemic and hypoxic conditions, gill damage and impaired osmoregulation (McFarland et al., 1999; Nussey et al., 2002).

In polluted environments, fishes are continuously exposed to ambient Zn in both the water and the food. Many recent studies of rainbow trout have indicated changes in metal uptake and physiological processes after Zn exposure (Chanchal and Prasad, 1992; Kakuno and Koyama, 1994; Nair and Suryanarayanan, 2000; Clearwater et al., 2002; Niyogi and Wood, 2003).